The Problem of Sex Determination in Dinophilus gyrociliatus.

Part 1.—The Sexual Cycle.

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With Plates 30–34 and 5 Text-figs.

Contents.

1. Introduction.

The problem of sex-determination in Dinophilus gyro-ciliatus is concerned, first, with the investigation of the factors involved in sex-determination in the sexual or fertilised egg; and secondly, with those involved in sex-determination of the parthenogenetic egg. In these two conditions the factors seem different, and for this reason are perhaps better considered separately. In the present paper only those concerned in sex-determination in the sexual egg are dealt with; while those involved in the parthenogenetic cycle, if it really exists, are left for a future paper.

In D. gyrociliatus parthenogenesis is only brought about by experimental means, that is by cutting out the male eggs from the capsules, inside of which they have been deposited in company with the female eggs.
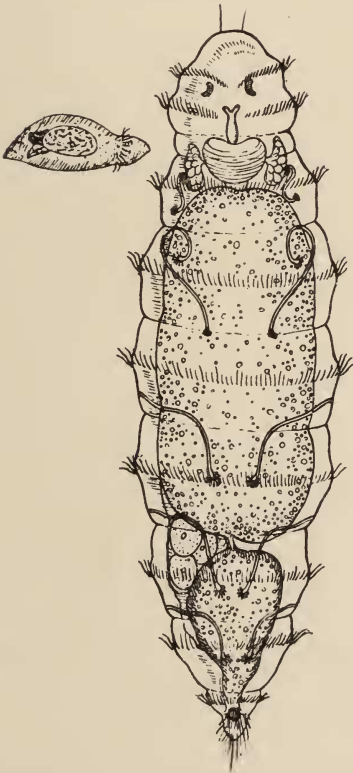
As early fertilisation of the female before she leaves the egg-capsule is invariably the rule in this species of Dinophilus, parthenogenetic development can only take place in the complete absence of the male. This I believe never takes place naturally, for during the course of three years' work I have not met with an egg-capsule that did not at least contain one male in addition to the female eggs. The proportion of three female to one male egg, or somewhat less than three to one, is remarkably constant, as shown by the table in the following section, which is made up from capsules collected at various seasons of the year. Parthenogenetic development, therefore, can only be brought about by the actual cutting out of the male eggs. Eggs in which early fertilisation has been prevented in this manner, after a certain period develop parthenogenetically, and the females to which they give rise in turn lay eggs of two sizes, male and female. The sex-determination taking place in the last, therefore, is that which I have called the sex-determination of the parthenogenetic cycle.

I have not, however, yet satisfied myself completely that I have succeeded in entirely eliminating the males in all these cases. This has to be done in the later segmentation stages to make sure of the matter. The extreme stickiness of the living egg, coupled with the small size of the male egg, render this a very difficult operation to perform. It is nearly always attended with failure. The small male eggs readily escape observation or adhere closely to the large female eggs and so make fertilisation possible at a later stage. For these reasons I feel considerable doubt if parthenogenetic development ever takes place, and I wish to reserve my final opinion on this subject till I have tested it by a larger number of experiments.

In one or two cases, however, where I have taken excep-

tional care, it seems almost impossible to believe that the males could have been left behind. It is on the somewhat scanty evidence of these instances, and the statements of de Beauchamp (**2**) that he was able to produce partheno- genetic development in D. conklinii after the exclusion of the males, that I have based the above statements of the occurrence of this con- dition. If parthenogenetic development does take place, then it plays no part in the usual life-cycle, but is only brought about, as I have said, by the artificial exclusion of the males under experi- mental conditions. Among Rotifers, in addition to the sexual cycle there is a par- thenogenetic one; in each sex- determination takes place, and there is no reason, therefore, why we should not find the same thing in Dinophilus, which all recent morpho- logical work has shown to be closely related to the Rotifers.

Text-Fig. 1.

Dinophilus gyrociliatus. Rudimentary male and full- grown female. The female shows the broken nature of the ciliated bands in the head region and the soleno- cyte-bearing nephridia.

The present work, from the first, was directed along the line of studying the influence of the sperm on the early development of the oögonial cells, for the reason that almost the first observation made was that of seeing the small males actively copulating with the females within the egg- capsule, just as these were about to hatch. This is readily

observed by placing a capsule in a drop of sea-water on a slide under the low power of the microscope. Through the transparent wall of the capsule the males can be clearly seen moving about and copulating with the females, and the actual injection of the sperm through the body-wall of the females witnessed. The small size of the young worms and their transparent condition render it even possible to follow the sperm as they leave the testis and pass down the penis and finally lodge in the body of the female; further observation revealed the fact that this early fertilisation invariably takes place, and that it is impossible to find a free-swimming female, either small or full grown, that has not been fertilized in this manner. Moreover the males seldom leave the egg-capsule; after the females have hatched, they remain about them and die shortly afterwards. The males never live a true, free existence, being without mouth or digestive system, and subsisting during the short course of their life on a small quantity of yolk substance in their interior. They are sexually mature at the time of hatching, and those I have kept under observation after fertilizing the females died in the course of a few days.

Starting with this observation of early fertilisation, it was easy to follow the course of events and observe what influence the sperm had on the female germ-cells as soon as these were distinguishable. It is not for some days after the females have left the capsule that the rudiments of the female cells appear, and this varies greatly with conditions. If the females do not obtain food their growth remains practically stationary, and after many weeks they will frequently be found in much the same condition as they were at the time of hatching. If supplied with proper food, however, they grow rapidly, and in four or five days the first rudiments of the germ-cells are distinguishable under the gut at the point of junction of stomach and intestine. At the time of hatching this triangular region is filled with a mass of clear structureless tissue, in which the mass of sperm received from the male collects, as shown in fig. 25. At the time the female

cells first begin to appear the female has grown very much, and since hatching has increased ten or fifteen times in size. The germ-cells themselves are first distinguishable as very minute refractive nuclei surrounded with a small amount of cytoplasm in the upper part of the mass of transparent tissue already mentioned, this mass of tissue being in short a primitive ovary. I have been able to determine very little regarding the exact origin of the germ-cells beyond the fact that they do not arise from the endoderm cells of the gut as Von Malsen (**8**) has suggested. The ovary is surrounded by a firm membrane, which is shown at a later stage in Text-fig. 3, and it is necessary to assume that if the primitive germ-cells arise from the gut wall, then they have to pass through this membrane in order to take up their final position. Von Malsen (**8**), however, was unaware of the presence of this membrane, which has only recently been demonstrated by Nelson (**11**) in Dinophilus conklinii. Moreover, the germ-cells are never seen arising from the gut wall, but are always within this membrane.

Once the female germ-cells have appeared their subsequent growth and rapid multiplication can be readily studied and their relationship to the sperm injected into the ovary at the time of hatching watched. It may perhaps add to the clearer understanding of the following paper if I briefly outline what I think takes place, and then describe the various stages of the process in detail later.

To recapitulate, then, briefly, the two varieties of eggs, male and female, are normally laid together in one capsule, but they develop immediately without the presence of the male, and are therefore fertilised inside the body of the female, or else they develop parthenogenetically. That they are fertilised in the female the subsequent observations show. In a few days the small male egg gives rise to the rudimentary male, which is full grown and sexually mature (Text-fig. 1) at the time the females are ready to leave the capsule.

The female, on the contrary, when she leaves the capsule, is very small and still in the larval state (figs. 6, 5, 4), the cilia-

tion and arrangement of the segments being quite different from that in the full-grown condition, which is only attained after a considerable period. The eggs may be clearly examined within the capsule as segmentation and development proceed. The development is direct, and as the time for hatching approaches the young larval females are seen to spin round within the capsule. This denotes that they are about to hatch and leave the capsule and commence their free existence. If the capsule is placed under the low power of the microscope at this stage and carefully observed, it will be seen, as I have already said, that the little males are actively copulating with the small females. Every female as she passes out of the capsule is seen to carry a small mass of sperm, collected under the gut at the junction of stomach and intestine at the point where the ovary will subsequently appear (figs. 4 and 25).

Examination of immature free-swimming females always shows them to be fertilised (figs. 5 and 6). If they are carefully fixed and sections cut from them at this stage, it will be seen that the female germ-cells are not differentiated, and although a mass of sperm is collected at the point where the ova will subsequently appear, no trace of the ova can be detected (fig. 25). They only appear at a later date, when the female has grown considerably in size. They are then seen as a few small refractive cells when examined in the living state, and as small nuclei surrounded with hardly any cytoplasm in the stained condition lying beneath the gut and amongst the mass of sperm (fig. 20). Shortly after they appear it is seen that each one is joined by a spermatozoön, the head of which has become embedded or attached to its nuclear wall, so that ultimately the nucleus of each primitive ovum is seen to be composed of one part derived from the spermatozoön and the other part derived from the egg (figs. 20 and 24). These two elements of the nucleus never fuse, but retain their individuality throughout all subsequent oögonial growth (fig. 29). The double nucleus divides amitotically, each half separately (figs. 16–19). In the majority of the divisions the

male and female portions of the nucleus divide equally, so
that a similar quantity of nuclear material, both male and
female, gets into each daughter-nucleus. There are probably
about forty to fifty divisions in all. In these the male and
female parts of the nucleus divide and move apart, the male
portion usually dividing first (fig. 35). Now and again, how-
ever, the female half of the nucleus seems to divide before
the male portion, so that the male portion gets left behind
and is shut off entirely in one of the daughter-nuclei (fig. 33).
Therefore, of the two resulting nuclei of this division, one
has the whole of the male part of the original nucleus and its
share of the female portion, while the other has only half the
female and no male substance. This appears to be the sex-
determining factor in the sexual or fertilised egg; for of
these two daughter-nuclei, the one that has received the
whole of the male element plus the female element becomes
the nucleus of a female egg, while that which has received
the egg portion becomes that of the male. Both these kinds
of eggs, once the sex-determining division has taken place,
grow rapidly (fig. 42). They seem to accomplish this through
the power of absorbing all the other immature egg-cells with
which they happen to come in contact, and in which the divi-
sions of the two portions of the male and female substance have
been equal. The outcome of this process is that the male-pro-
ducing egg is not fertilised while the female-producing egg is.
It is, however, impossible to speak in the strict sense of the
word of the male egg as unfertilised, as it has been directly
under the influence of the sperm in all the early oögonial divi-
sions previous to the sex-determining one. For all the primi-
tive germ-cells are joined in the first place by a spermatozoön,
irrespective of the fact that only some of these will give rise
to ova later, and that the majority will be only nurse-cells.
It is only in the late stages, shortly before the female egg is
laid, that the two portions of the nucleus, the male and
female, actually fuse beyond recognition. As the two kinds
of eggs, male and female, are not found in the simple ratio,
but in the proportion of three or two females to one male, it

is probable that some other division takes place in the case of the female egg. This ratio, however, varies widely, as the table in the following section will show, and according to Von Malsen (**8**), it can be greatly altered by conditions of heat and cold. I have been unable to obtain any light on the subject from my sections. It is impossible to follow the above described cytological changes on living material.

2. Material, Habits, Methods, etc.

The group of primitive Annelids, represented in the genus Dinophilus, comprises some eight or nine species. They are remarkable for the fact that some show a well-marked sexual dimorphism, in which the male is rudimentary, without any mouth or digestive tract, while in others the sexes are the same size and exhibit no signs of this dimorphism. The group as a class, therefore, is readily divisible into two subdivisions, in one of which all the species are sexually dimorphic and unpigmented, while in the other they are an orange red in colour, and sexually monomorphic. The former may be called the Leucodinophilidæ, while the latter may be called the Erythrodinophilidæ. The known species, many of which are of doubtful specific value, may be arranged under these two subdivisions, as follows:

Leucodino-
philidæ
1. Dinophilus gyrociliatus, Schmidt, 1857·
2.   ,,   apatris, Korschelt, 1882.
3.   ,,   conklinii, Nelson, 1907.
4.   ,,   metameroides, Hallez, 1879.
5.   ,,   pygmæus, Verrill, 1892.

Erythro-
dinophilidæ
6.   ,,   vorticoides, Schmidt, 1848.
7.   ,,   gardineri, Moore, 1899.
8.   ,,   tœniatus, Harmer, 1889.
9.   ,,   gigas, Weldon, 1886.

Of the Leucodinophilidæ the first three species, D. gyrociliatus, D. apatris, D. conklinii, are closely related,

and are probably one and the same.   The form on which the following work has been done is one of these three species, though exactly which I have been unable to decide.   I have placed it under the head of D. gyrociliatus, as this is the oldest of these names.   Figures of the male and female, drawn to scale, are shown in Text-fig. 1.   It will be seen that the female is very much larger than the male, and that the sexual dimorphism is well marked.

The species D. gyrociliatus was established by Schmidt (**15**) from material obtained from the old harbour at Naples and described by him in 1857.   He gave a short description, with one figure, from which it is not possible to gain very much information.   It was subsequently studied by Meyer (**9**), who gives a figure of it in his studies on the embryology of Annelids.   At about the same time it was made the subject of a lengthy paper by Repiachoff (**13**), who described its anatomy and who first found the male form.   It now appears through the great extension of the harbour at Naples, and the consequent contamination of the sea-water, to have forsaken its old habitat in the Porto Vecchia, for during the last ten years I have repeatedly looked for it during different visits to Naples, without being able to find it.   It was formerly found in abundance on the masses of Bugula attached to the piers of the old harbour.   Recently I have often examined the Bugula growing there, without however ever in any case observing Dinophilus.

I am unable to say, therefore, from personal experience, if the species obtained at Plymouth, and on which the present work has been done, is the same as D. gyrociliatus of Naples.   Prof. Meyer, of Kasan, however, has kindly examined some material that I have sent him, and he tells me that he find it very similar, as far as he can determine from preserved material, to D. gyrociliatus, with which he was formerly familiar at Naples.

The other species with which it may possibly be identified is, first, D. apatris, described by Korschelt in 1882, from a salt-water aquarium at Freibourg.   It has been suggested

by Repiachoff (**13**) that this species is identical with D. gyro-
ciliatus, and its specific value seems somewhat doubtful.
No one who has worked on D. gyrociliatus has ever
worked on D. apatris, so it seems impossible to determine
its real value. I have sent material of my species to Prof.
Korschelt, who has kindly examined it for me, but has been
unable to report anything further than that it is undoubtedly
closely related to D. apatris. In the last few years this
species has been made the subject of considerable investi-
gation by Von Malsen (**8**) in Prof. Hertwig's laboratory, and
a special variety has been described under the name
of "D. apatris forma tergestina," from Trieste, by
Stiasny (**18**).   The second species with which D. gyro
ciliatus may be related is the American form D. conklinii.
With this species the Plymouth variety has possibly less
relationship than with D. apatris. Dr. Nelson (**10, 11**),
who has made D. conklinii the subject of two extensive
memoirs, as well as Prof. Conklin (**3**), who has done con-
siderable work on the oögenesis, have kindly examined
material of the Plymouth species for me, and they both
report that they consider it different from their species.
M. de Beauchamp also tells me he thinks the Plymouth
species different from the form he has investigated at Roscoff,
which he considers the same as D. conklinii.

The females of D. gyrociliatus at Plymouth are very
worm-like in their general appearance (figs. 1, 2, and 3.)
The body lengthens out very considerably in the usual swim-
ming attitude, then measuring quite 1·5 mm. in length.
While at rest it is more contracted and the segmentation
more pronounced. The head is blunt, conical, and rounded
in front, and narrows into a constricted neck region, where
it joins the trunk. This region, however, in D. gyro-
ciliatus is not so marked as in the allied species
D. conklinii. The ventral surface of the head is very
much flattened, while its dorsal surface is rounded, and bears
a pair of red crescentric eyes. The two first trunk segments
are much bigger than in D. conklinii, where they are some-

what reduced; the caudal appendage or last segment is sharply marked and is conical and more pointed.

Like other members of the group, the female of D. gyrociliatus is encircled by a number of ciliated rings, nine in number, of which two that are interrupted dorsally belong to the head, while seven belong to the trunk region. The ninth band is on the caudal appendage and is much reduced. In addition to the ciliated bands, the ventral surface is extensively ciliated in the pre-oral and oral region, and then running back along the entire ventral surface is an extensive tract that gradually narrows as it approaches the caudal segment, where it is much restricted. The ciliated bands are narrow and furnished with relatively few but very powerful cilia. Those of the trunk are narrow transverse bands, that are uninterrupted on the dorsal surface; each band encircles the middle of the segment. The two bands of the head, as in D. apatris and D. conklinii, are interrupted dorsally and relatively wider than those of the trunk. In addition to the ciliated tracts and bands, D. gyrociliatus possesses conspicuous sense-hairs, two of which are arranged symmetrically, on the anterior surface of the head, and are undoubtedly tactile in function. There are five pairs of solenocyte bearing nephridia, whose arrangement can be best seen in Text-fig. 2 and figs. 3 and 5.

The males are small and very inconspicuous relatively to the females, and, as I have said, are seldom seen outside the egg-capsule. Korschelt (6) has also remarked on the fact that in D. apatris the males are rare and live only a few days, and Hallez (5) has mentioned that it is a singular fact that the males of Dinophilus are never found in company with the females. Their shape is subject to considerable variation dependent on their state of contraction. It roughly agrees with that of the males in D. apatris and D. conklinii. When moving rapidly their shape is much as that shown in fig. 10, while when at rest it is as in figs. 8 and 11. When moving about slowly within the egg-capsule it is more like that of figs. 7, 14, and 15. About the anterior end of the

male there is a ring of cilia, which is continuous on the ventral surface with a strip of fine cilia covering the whole of this surface (fig. 10). In the interior of the body little can be distinguished but a mass of granular yolk, which apparently

TEXT-FIG. 2.
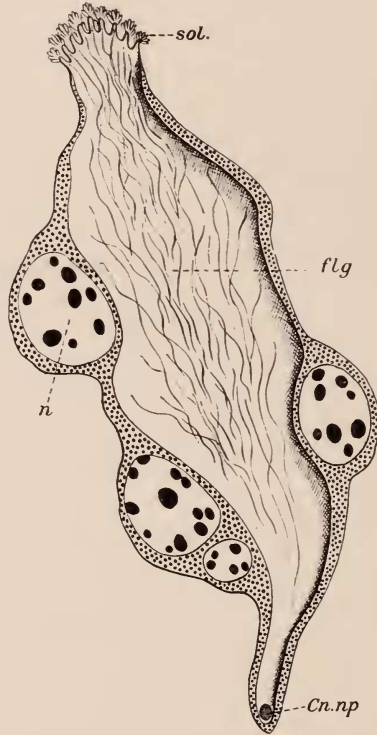
Nephridia of full-grown female drawn from living preparation after impregnation with methyl blue. *Cn. np.* Nephridial canal. *flg.* Flagella. *n.* Nucleus of canal wall. *sol.* Soleno-cytes.

serves as food during the short existence of the male. There is no mouth, stomach, or gut. In the posterior region there are well-developed seminal vesicles crowded with sperm; the ducts of each vesicle join to form a common ejaculatory duct

that leads down a short penis, which, except during the act
of copulation, is kept retracted within the penis sheath.

I have been unable to distinguish whether the cilia of the
anterior end are inserted in a well-marked groove as in D.
conklinii. They are not so sharply limited to a ring or
circle, but seem to cover almost the whole of the anterior end
as shown in fig. 11. This appearance, however, varies very
much with the state of contraction of the male, as in fig. 7 ;
where the end is extended the cilia appear as a sharply limited
circle.   There is also this further difference between the males
of D. gyrociliatus and both D. apatris and D. conklinii,
in that the testis or the vesicula seminalis is distinctly
paired as shown in figs. 8, 11 and 14. This condition does
not seem to hold in D. apatris, the males of which have
been carefully figured by Korschelt (6).

If the young females that have just left the capsule are
examined in the living condition, under an oil-immersion lens,
it will be seen that the ciliated rings in the trunk, like
those of the head region in the adult condition, are interrupted
dorsally and are incomplete. The whole of the dorsal surface
between the rings is also covered with fine very short cilia,
and the female has a characteristic larval appearance. The
relative size and configuration of the segments and general
shape is also different from that of the adult condition.
In the young female the head region is by far the widest part
of the body, so that the animal seems to taper from the head
towards the tail, and looks not unlike an attenuated wedge in
shape, the thick end of the wedge being the head (Text-fig. 4).
In the resting position shown in fig. 6 this shape does not
show, but only appears during the act of swimming or moving
rapidly.

I first concluded that these females, of this peculiar shape,
were the male forms of the species, for whenever I examined
one of them under the microscope, I invariable found it to
have sperm beneath the gut. I concluded therefore from this
that I had found a new species of Dinophilus in which the
females were much the same as in D. gyrociliatus, D. apa-

tris and D. conklinii, while the males were simply much
reduced in size and not rudimentary (without mouth, stomach
and gut), as in the species just mentioned. I was confirmed
in this supposition by the fact that these males in some jars
never changed or grew any bigger, and as they contained
active live sperm, they must therefore, I thought, be full grown
adults. Now the fact that the females may remain indefi-
nitely in the immature larval condition, to which I have already
called attention, seems to depend on certain factors in the
environment of which the presence of food only forms one.
It so happened in the two jars from which I started all my
subsequent cultures, one at the end of six months was swarm-
ing with large females, while the other was full of what I
thought at the time were the males. In this jar not a single
large female was to be seen, nor did any appear after several
months, although I supplied these small forms with abundant
food. I was able, however, to demonstrate later that they were
immature females, by taking a few of them and putting them
in another dish, in which the conditions were somewhat diffe-
rent, for they then grew rapidly into the large females. I was
forced to conclude, therefore, that these small forms were not
the males, and obtaining some eggs about the same time, I
saw, as these hatched, the real males.

At Plymouth D. gyrociliatus is never found in the rocky
tidal pools in company with the red species D. tœniatus, and
my material was first obtained from some sandy dredgings
from Cawsand Bay, one of the smaller divisions of Plymouth
Sound. This material had been allowed to stand in the labo-
ratory for some months before it was noticed that it was
swarming with D. gyrociliatus. I introduced it into one
of the large laboratory tanks from this jar. There it soon
established itself and has bred for the last three years. In
this tank it has never become very abundant, although in
smaller culture jars it sometimes increases in great numbers,
without any very obvious reason. I have been unable to find
out to what condition this sometimes sudden increase is to be
attributed. Its food consists of various small algæ, Pleuro-

coccus, Foraminifera, and, perhaps most important of all, various Naviculæ and other bottom species of Diatoms. When supplied with these in abundance, however, it does not always increase and become numerous. There seem to be some other conditions that I have been unable to discover to account for this increase in numbers besides that of food. In the large tank in the laboratory, its numbers remain very much the same throughout the different seasons of the year.

TEXT-FIG. 3.
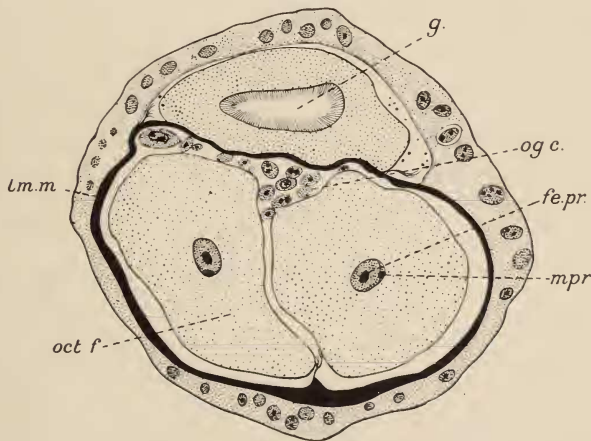
Transverse section of large female in the region of the ovary, showing several female eggs. Limiting membrane of ovary shown in black. *fe. pr.* Female pronucleus. *g.* Gut. *lm. m.* Limiting membrane of ovary. *og. c.* Oögonial cells. *oct. f.* Female egg. *m. pr.* Male pronucleus.

At times it almost disappears, but always reappears again shortly afterwards, in somewhat increased numbers. This tank is connected with the general water circulation throughout the laboratory, but the worms have not spread to the other tanks. If a white porcelain plate or dish is placed for some hours on the side or bottom of this tank all the Dinophilus in the tank will soon collect on it, and this affords a ready means of obtaining them in numbers. In this manner almost every individual in the tank can sometimes be withdrawn. They never show a tendency to collect on the glass side of the

tank nearest the source of light, although collecting so readily on any bright object introduced into the tank itself. It breeds continuously through the winter and summer months, and those I have kept under observation from day to day lay their eggs in several, sometimes as many as five, batches. This takes place as the large ovarian eggs ripen. There is sometimes a considerable time, as much as a week, between the laying of one batch and the growth of a fresh lot of eggs. Once a female has got rid of all its eggs, the germ-cells seem completely used up and no more appear and the animal dies.

The females with eggs seem liable to some disease or infection that causes them to swell up and become dropsical, the cuticle all over the body becomes puffed out in blebs containing fluid, and the animal soon dies. In this state they seem incapable of laying their eggs, and when once this disease appears in a culture jar it rapidly spreads and destroys all the females.

My mode of procedure in order to obtain eggs in any numbers has been to take half-a-dozen two-litre jars of sea-water and place a number of females with eggs in each and supply them with proper food. The jars are set aside for a month or so, at the end of which time it will usually be found that one of the jars at least will be crowded with Dinophilus, while possibly the others will contain none.

The manner in which the egg-capsule is formed is somewhat peculiar, and recalls the way it is accomplished in many of the Turbellaria. The female about to lay eggs contracts into a round mass and pours out a copious secretion from the large mucus cells of the cuticle, which are especially numerous in the region of the ciliated bands, as shown in fig. 2. In this manner the beast is surrounded with a thick coat of mucus (fig. 12). The eggs are then passed out through a small median pore on the animal's ventral side somewhat anterior to the anus. When all the ripe eggs have been laid, the animal crawls out of the capsule, with which it has surrounded itself, leaving the eggs behind. The mucus after a time seems to harden on contact with the sea-water,

but still remains very sticky on the surface, so that if it is touched with a needle it adheres so firmly to both needle and the bottom of the dish that it usually tears apart and the eggs in the interior are destroyed. The eggs themselves are also difficult to handle in the living state, as they stick with great tenacity to the inside of pipettes and sides of needles, and cannot be detached without breaking. I have been reduced to handling them when stuck to small pieces of tissue paper. Small pieces of paper are cut, and then with the aid of fine forceps the egg-capsule is scooped up, the capsule adhering firmly to the paper ; then the paper with the capsule may be readily transferred from one dish to another, or into the fixing fluid.

The two kinds of eggs, male and female, are laid together (fig. 57) in a fairly constant ratio of a little more than two female eggs to one male, and this ratio does not appreciably change during the different seasons of the year. I have made, however, no special observations to test this point. The following table gives the number of eggs, male and female, counted in capsules collected at different seasons of the year :

Table showing the Proportion of Male to Female Eggs in Forty Capsules collected at Different Seasons of the Year.

Spring.					Summer.		
1 Capsule	2 ♂	4 ♀	.	11 Capsule	2 ♂	5 ♀
2 ,,	1 ,,	2 ,,	.	12 ,,	2 ,,	8 ,,
3 ,,	2 ,,	6 ,,	.	13 ,,	4 ,,	8 ,,
4 ,,	2 ,,	8 ,,	.	14 ,,	1 ,,	2 ,,
5 ,,	2 ,,	6 ,,	.	15 ,,	2 ,,	6 ,,
6 ,,	2 ,,	4 ,,	.	16 ,,	3 ,,	6 ,,
7 ,,	4 ,,	10 ,,	.	17 ,,	2 ,,	5 ,,
8 ,,	2 ,,	5 ,,	.	18 ,,	1 ,,	3 ,,
9 ,,	2 ,,	8 ,,	.	19 ,,	3 ,,	8 ,,
10 ,,	1 ,,	3 ,,	.	20 ,,	2 ,,	6 ,,
	Total 20 ♂	56 ♀			Total 22 ♂	57 ♀

Autumn.						Winter.				
21	,,	1 ♂	3 ♀	.	31	Capsule	3 ♂	8 ♀
22	,,	2 ,,	6 ,,	.	32	,,	1 ,,	2 ,,
23	,,	3 ,,	8 ,,	.	33	,,	1 ,,	3 ,,
24	,,	4 ,,	10 ,,	.	34	,,	1 ,,	2 ,,
25	,,	2 ,,	3 ,,	.	35	,,	1 ,,	2 ,,
26	,,	4 ,,	8 ,,	.	36	,,	3 ,,	6 ,,
27	,,	2 ,,	5 ,,	.	37	,,	1 ,,	3 ,,
28	,,	1 ,,	3 ,,	.	38	,,	1 ,,	2 ,,
29	,,	3 ,,	6 ,,	.	39	,,	4 ,,	10 ,,
30	,,	1 ,,	3 ,,	.	40	,,	2 ,,	5 ,,
	Total	23 ♂	55 ♀			Total	18 ♂	43 ♀

Dinophilus is a remarkably difficult animal to fix well, as even the most rapid of penetrating fixatives allows of considerable shrinkage before it can act. In this respect D. tœniatus is even more difficult to handle than D. gyrociliatus. I am inclined to attribute this to the fact that the animals are surrounded by a thin layer of mucus of an oily nature that effectively prevents the rapid penetration of the fixing fluid. As my interest in Dinophilus lay entirely in the direction of obtaining good fixation of the ovary, germ-cells, and ova, the fixatives I have used have been judged entirely by the results they have given with regard to these alone, and for this reason they may have given indifferent fixation of the tissues in general.

The large female eggs are yolky and granular, and on this account crumble and break readily in sectioning. I have for this reason tried to modify all my fixatives with the object of avoiding this as much as possible. This I have done by the addition of a small quantity of HNO_3 to my solutions, which renders the eggs much less brittle. The fixatives that on the whole have given the best results are sublimate-nitric, or acetic, and Perenyi's solution, the sublimate solution consisting of a saturated solution in either sea-water or distilled water, to which 2 per cent. nitric acid has been added. The best results were obtained by using the sublimate boiling hot,

when a solution in distilled water was employed. There is little difference in the results obtained with sublimate-acetic or nitric, except that in the latter the sections are not so granular. I have made a very extensive trial of all the good fixatives in an endeavour to improve on the above reagents, but without success. A fixing fluid that has given fair results next to sublimate-nitric is a slight modification of Eisen's iridium chloride solution. All the osmic and picric acid mixtures give but medium results, especially the picric acid ones, which are invariably poor. In the following work, therefore, I have relied almost entirely on the results obtained with sublimate-nitric and acetic, and Perenyi's solutions.

There is considerable difference in the histological appearance of a section fixed with sublimate-nitric and that with Perenyi's solution. The general appearance of the section is much better in that fixed with Perenyi, while the histological details are shown much clearer in that fixed with sublimate. This is shown by the comparison of figs. 21 and 32 with fig. 42. Figs. 21 and 32 are from sections of material fixed in sublimate-nitric, while fig. 42 is from Perenyi's solution. It will be seen that although the section drawn in fig. 42 appears in general better fixed than that of fig. 32, there is nevertheless more shrinkage of the nuclear details in fig. 42 than in that of fig. 32, and this is more marked on comparing the actual sections themselves.

A reagent that I have found most useful in investigating the early oögenesis is aceto-carmine. With the use of this fixative and stain, it is possible to follow the early process of fertilisation from a whole preparation with great convenience. Figs. 13, 20 and 24 are drawn from such preparations.

In sectioning the eggs I have found the most convenient method is to cut them, when fastened, to bits of amyloid liver. Another method consists in allowing some hard paraffin to cool in a watch glass and then cutting a small hole in this; the eggs, which are in zylol or cedar oil, are then carefully transferred into this with a fine pipette and the excess of oil got rid of as much as possible. The paraffin is

then slowly melted, and after remaining melted long enough
to penetrate the eggs is cooled again. The eggs in the
meantime remain close together in a heap and can be after-
wards cut all in one small block. On the whole I have
obtained the most satisfactory results with the first method,
i.e. of attaching the eggs to a bit of liver with albumen,
tiresome as this operation is to perform.

3. Fertilisation and Early Oögenesis.

If an egg-capsule about twelve days old is examined under
the low power of the microscope, it will usually be seen that
the young worms within it are about to hatch. The females
are distinguished by their size and the conspicuous red eye-
spots. At this stage they are curled up, and if ready to
hatch, are revolving slowly within the egg membrane. The
males are not so conspicuous on account of their small size
and their lack of any marked conspicuous anatomical
feature. Gradually the movements of the female become
more and more vigorous, until she finally frees herself from
the egg membrane. At this moment she is usually joined by
one of the males, which immediately attaches itself to her so
closely that after a moment or two it can hardly be dis-
tinguished. If it had not actually been seen to join her, it
would be almost impossible to tell that the small dot on the
side of the female was not merely a fold of the body-wall.
If the male is then closely watched, it will be seen to contract
strongly several times in succession, and this corresponds
with the injection of the sperm. If the conditions for observ-
ing the act of copulation are favourable, it is possible to see
the sperms as they pass out of testis or vesiculæ and down
the short penis and lodge finally within the body-wall of the
female. All this time the female is moving rapidly within
the egg-capsule, and almost as fertilisation takes place
usually succeeds in forcing its way out of the capsule. The
moment after the male has contracted in the act of discharg-
ing the sperm, it becomes detached and remains behind in

the egg-capsule as the female passes to the exterior.   It is, however, possible for the same male to attach itself to the next female and so in turn fertilise her.   In this manner I have seen a male fertilise three successive females as they were leaving the capsule.   In the case of the third, it was carried some little distance outside before it finally succeeded in accomplishing this; it then detached itself and slowly returned to the capsule, adhering motionless to it until it died in a few days.

It is remarkable that the males are perfectly quiet within the capsule until the females start moving.   Then they seem suddenly thrown into a state of great excitement and move about restlessly until they come in contact with the females. Immediately fertilisation is accomplished they become quiet

TEXT-FIG. 4.

Young fertilised female, showing the outline and shape during swimming.

again and remain motionless.   Under the microscope, if the capsule happens to be compressed by the cover-glass and the normal conditions thus disturbed, the male seems occasionally to have some difficulty in forcing its penis through the body-wall of the female, and in so doing the sperms are sometimes discharged into the water.

The ovary in general structure and position resembles that of D. conklinii and D. apatris.   It occupies the concavity on the ventral side of the alimentary canal formed by the junction of the stomach and intestine.   It is usually crowded with egg-cells, which push the intestine to one side.   The ovary consists essentially of three parts, the oögonia, the oöcytes, and the peritoneal sac enveloping the whole.   The oögonia form a mass of closely packed round cells in the anterior region and send two lateral horns towards the head. The oögonial cells graduate in size as they proceed back-

wards until they reach the oöcyte stage. In the oögonial region the cells are seen rapidly dividing, while in the oöcyte region they are seen fusing together to form the large female and the small male eggs. The oöcytes or ovarian eggs fill up the greater part of the ovary, and crowd the oögonia laterally and ventrally. The largest oöcytes are found in the posterior part of the ovary. The peritoneal investment of the ovary consists of an extremely delicate epithelium which encloses the oögonia and oöcytes as in a

TEXT-FIG. 5.
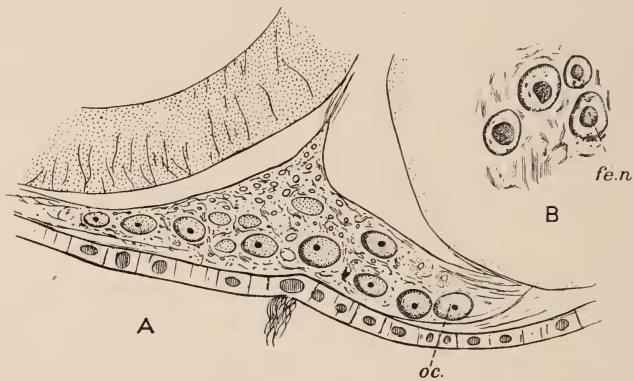
A. Rough outline drawing of ovary of young female in which fertilisation has been avoided. The sperm are not present in oögonial cells. B. Enlarged view of a few of the oögonial cells, showing only one chromatic body, the sperm chromatic body being absent.

bag (Text-fig. 3, *lm. m.*). This investment sends strands to the body-wall and the alimentary canal (fig. 42).

In the larval female leaving the egg-capsule the condition of the ovary is very rudimentary. It consists of a small triangular mass of tissue, filling the space between stomach and gut. It is very difficult at this stage to make out its structure. In whole preparations stained with aceto-carmine no cell outlines or nuclei can be seen. It seems to consist of a perfectly clear homogeneous mass of cytoplasm, uniformly finely granular throughout. In the living condition, certain

of these granules seem somewhat refractive and larger than the others. They appear to be the forerunners of the germ-cells, but at this stage they can hardly be said to differ from the ordinary cytoplasmic granules. In the middle of the ovary, collected together in a small mass, are the sperms, which in the living condition are actively motile. At a stage somewhat later than that shown in fig. 25, a few of the granules already mentioned are seen to have enlarged, and are the first definite oögonial cells. As far as I have been able to determine, these arise anywhere in the ovarian tissue and not in a more or less restricted portion of it, as in D. vorticoides and D. tœniatus. Once the oögonial cells appear, they increase very rapidly in numbers and assume a characteristic round shape. They have large darkly staining nuclei, surrounded in the early stage with hardly any cyto-plasm. They soon fill up the whole ovary, but are most numerous in the anterior region, where they seem to be undergoing very rapid proliferation. At a slightly later date it can be seen that they are dividing solely in this anterior region, while towards the middle and posterior region they are not dividing, but are increasing in size, so there is a graduation in size of the oögonial cells from the minute almost granular condition at the anterior end, to the large cells that are almost oöcytes in the posterior region. This stage is shown in Text-fig. 2, which is drawn from a com-pressed aceto-carmine preparation; therefore the cells are forced out of position and their relative position distorted.

With the appearance of the oögonial cells and their rapid multiplication, the sperms, which have formed a compact mass up till this time, become dispersed throughout the organ, a large number being pushed up in the angle between stomach and gut (fig. 9). If an aceto-carmine preparation is made of a female at this stage, one sees a picture something like that shown in fig. 20. This represents a preparation of the ovary compressed under a cover-glass, as seen under an oil-immersion lens. Inside the limiting membrane the organ is seen crowded with sperm. The female germ-cells are still

few in number, and have large granular nuclei, each sur-
rounded with a thin layer of cytoplasm. Close examination
shows that every female cell has been joined by a sperma-
tozoön. The different parts of these, the heads and tails,
can be clearly distinguished. There are apparently many
more sperm than female cells, so that the ovary contains
many lying free and unattached. On making a preparation
of another female, somewhat more advanced than the one we
have been considering, we get the condition shown in fig. 24.
Here the process has gone a step further, and the primitive
oögonial cells have increased appreciably in size. There,
attached sperm-heads are visibly undergoing absorption.
The tails of the sperm have partially disappeared, but in
several instances can still be distinguished. The sperm
heads are lying within the cell wall, or are in the act of
fusing with the nuclear wall of the oögonial cells. In a
slightly later stage still, all the sperm heads are in the cyto-
plasm or have definitely fused with the nuclear wall of the
oögonia. During these stages the sperm head remains a
compact mass of darkly staining chromatin.

Lastly they come into definite relation with the nucleus of
the oögonial cell, so that this consists of two separate parts,
each with its own nuclear wall, surrounded by one main
nuclear membrane. This condition is shown under high
magnification in fig. 34; and in a later stage again in fig. 29.
The nucleus of the primitive oögonial cell, therefore, is of a
double character : one half, the smaller, contains the male
substance which has been derived from the sperm head,
while the other, the larger, contains the female. These two
parts retain their individuality throughout the growth period.
In all the subsequent nuclear divisions these two parts of the
nucleus divide amitotically (figs. 16, 33, 34). In none of
these divisions does the male or female substance form indi-
vidual chromosomes, but each divides directly into two equal
parts. As these masses pull apart, the chromatic substance
seems to be drawn out into irregular digitations that give at
certain stages a false appearance of chromosome formation.

This appearance is quite irregular, however, and is simply the result of the rapid drawing apart of the two masses of chromatic substance. These bodies, in the case of the female chromatic substance, are figured in figs. 49, 51, 53, 54, 61, and 64. The same appearance is presented also by the male substance during division, but on account of its much smaller volume is not so distinct. The female substance is always crowded with peculiar yellowish refractive globules, shown in these figures by the white dots. They are also present in the male chromatic substance, but are not so numerous or so large as in the case of the female substance. They always show well in material fixed in sublimate-acetic; possibly not so clearly in material fixed in sublimate-nitric; and hardly at all in material fixed in Perenyi's solution. They seem larger in the early oögonial stage and become smaller as the oögonia increase in size. They disappear from the chromatic substance at the oöcyte stage. They would seem to correspond with the peculiar vacuoles that have been described under a number of names in the nucleoli of different oögonial cells. In Dinophilus they seem to be very similar to the refractive granules of the chromatic substance in Batrachoseps seen during spermatogenesis, as described by Eisen (4). They are small, of a light yellow colour, and do not stain with iron-hæmatoxylin. They are also doubtless of the same nature as the granules figured in the nuclei of the oögonial cells of some molluscs, by Obst (12), and in Pholcus by Van Bambeke (1). I shall call them the endochromatic granules, to use the term applied to them by Eisen (4).

In the following paper I shall refer to the two chromatic bodies of the oögonial cells, one of which is derived from the sperm and the other from the female, as the male and female pronucleus respectively. It must be understood, however, that while they may be spoken of as pronuclei, they are more properly one nucleus divided into two distinct compartments, each of which retains its own individuality, although surrounded by a common nuclear membrane.

Now if one of these pronuclei is derived from the sperm, in

the manner we have just seen, then the germ-cells of a female in which early fertilisation has been prevented, by the cutting out of the males in an early segmentation stage, should show only one of them within the nuclear membrane. Although this operation of cutting out of the males is a difficult one to accomplish, as I have fully explained in the Introduction, in the few instances in which I have succeeded, the oögonial cells of these females on development only showed one pronucleus, and are quite different from the ordinary oögonial cells with their two darkly staining masses of chromatin in each compartment of the nucleus. In Text-fig. 5 a drawing is shown of the germ-cells of such a female. The small oögonial cells in this figure, which is taken also from an aceto-carmine preparation, are about the same size as those shown under greater magnification in fig. 24. In fig. 24 the male and female portion of the nucleus show quite distinctly, while in Text-fig. 5A and B, the male portion, the sperm head is not present. In all the oögonial cells in which the sperms have been prevented from entering, we find therefore that the nucleus consists of one single compartment, with one chromatic body. The double character shown by the nuclei of the fertilised oögonial cell does not appear. In rearing these females, moreover, it is clear almost from the first that the conditions of growth are very materially altered. Their germ-cells appear much later than in the normal state, and grow very slowly, or hardly at all. In this particular instance they took over a month to attain the size shown in this drawing; in a normally fertilised female they would attain this size in a week's time.

I have stated in the Introduction that I am still doubtful as to whether females, in which early fertilisation has been prevented, can really be raised to the adult condition, that is to a stage when they lay eggs. While I think I have succeeded in one or two instances, I am by no means absolutely certain that I did avoid fertilisation in these instances. The females suffer a high rate of mortality after being cut out of the egg-capsule, so that it is necessary to make a great number of ex-

periments before any results can be attained. It is, however, a comparatively easy matter to get them to live to the stage represented in the above text-figure, and from this it is clear that the sperm part of the nucleus is missing. Comparison of Text-fig. 5B with fig. 24 brings out this point clearly. The stage, however, represented in this text-figure is much earlier than that at which any possible sex determination can take place in the unfertilised female.

This experiment confirms the nature of the small accessory nucleus of the fertilised oögonial cell, and shows that it is spermatic in origin. The fact also brought out by this experiment, that when the sperm are prevented from entering the oögonial cells, these grow slowly, and apparently take months to reach the stage they would normally attain in a few days under the influence of the sperm, shows the sperm to play an important rôle in the metabolism of the oögonial cells, that may even extend to the growth of the female in general. It is at least remarkable that many of the females in which early fertilisation has been prevented never attain anything like the size of the fertilised ones.

As I have said, the primitive oögonial cells seem to be dividing most actively in the anterior region of the ovary, while in the posterior region they seem to be increasing in size. The multiplication of the oögonial cells is so rapid, however, that the whole organ becomes crowded with them before those of the posterior portion, which are the oldest, have had time to grow very much (fig. 9). The result of this is that there is a stage in which all the oögonial cells are much the same in appearance and of approximately the same size. Each has been joined by a spermatazoön, the head of which has lodged in the cytoplasm (fig. 24). This stage is followed by one in which they increase in size, but as far as I can determine do not actively divide. When they have increased several times in size, their nuclei undergo a series of rapid divisions, not followed by any division of their cytoplasm. It is possible that these divisions are, at the same time, accompanied by some fusing together of the cells. At this point it

is difficult to make out exactly what does take place. What one sees in sections is that amongst the oögonial cells are some that are very large and yolky and have the appearance of being formed by the fusion of several primitive oögonial cells. They may have three, or as many as five nuclei, that seem to be actively dividing quite independently of one another, while the large mass of cytoplasm in which they lie shows no cell walls, and is distinctly yolky and granular in appearance, and very unlike the cytoplasm of the early oögonial cells. There are two possible ways in which these masses can be formed : first by the proliferation of the nucleus of the oögonial cell without any actual cell division ; or secondly by the fusion of several independent cells. I am inclined to believe that the first stage in the process is begun by the division of the nuclei, and that fusion only takes place secondarily, and that the two processes to a certain degree go hand in hand.

A number of these primitive egg masses are shown in the interior of fig. 21. Here one sees a large number of nuclei distributed throughout a small mass of yolky cytoplasm. In fig. 22 one of these masses is shown under greater magnification, at a somewhat later stage. Here we get a large number of actively dividing nuclei, arranged around the periphery of a mass of yolk. Examination shows that these nuclei are undergoing division, apparently quite independently of one another. It is clear that whether these masses are formed by the fusion of cells, or by the growth of a single cell through multiple nuclear division, the result would be the same. I have, however, been unable to determine exactly which of these processes takes place.

To go back to an earlier oögonial stage, a series of figures (figs. 16–19) represent the process of nuclear division at this stage. Fig. 16 represents a small oögonial cell, the nucleus of which is about to divide. Here it can be seen that the male and female portions divide simultaneously and amitotically. The female portion is much the larger and contains one endochromatic vacuole (*rfg.*). The male portion has already

divided and drawn apart. In fig. 17 a later step in the same process is shown from another section. Here the male portion (*m. pro.*) has already divided and moved apart, while the female portion has drawn out and is about to divide, as shown in the text-figure (fig. 18). Finally, in fig. 19 the two separate nuclei are shown. The darkly staining male portion is clearly distinguishable, while the female portion is represented by a number of chromatic granules distributed throughout the nucleus and not very clearly shown in the figure. It is thus seen that in all these nuclear divisions the male and female portions of the nucleus divide separately. In the majority the division of the male substance precedes that of the female. On referring to fig. 22, it will be seen that each female portion of the nucleus has the small portion beside it, within the same nuclear well. In most instances the male portion has already divided and is represented by two black masses which are separating. Similarly, in fig. 21, the male and female part of each nucleus can be distinguished. In fig. 28 are shown two nuclei which have just divided and separated, in a small mass of cytoplasm; here the structure of the nucleus is well shown. Fig. 29 represents a similar nucleus under greater magnification, in which the separation of the male and female portions is again shown. Fig. 35 represents still another series of nuclei; while in fig. 34 an early stage in the division of the male portion of the nucleus is shown.

4. Late Oögenesis and Formation of Male and Female Eggs.

As a rule the nuclei in such a mass as that shown in fig. 22 divide equally; that is, an equal portion of male and female chromatic substance goes into each daughter-nucleus. As long as these nuclear divisions continue to be equal no change takes place beyond that of slow growth. But now and again the divisions can be seen to be distinctly unequal, that is, for some reason or other the female portion has divided before the male, and this last has gone wholly over,

without any division, into one of the nuclei. In fig. 33 such a division is shown; here two egg masses lie side by side, in one of which, that on the right, the male and female substance has divided equally, while that on the left shows the male portion entirely on one side. The examination of the foregoing and subsequent sections of the series shows no other portion of the male nucleus present. Similarly in fig. 31 we get the same condition. Here in one nucleus the male substance is entirely on one side. In the larger oögonial masses, such as those already discussed in fig. 22 and fig. 35, similar divisions take place. I believe this division to be the sex-determining one. The nucleus which has received both male and female substance gives rise to the large female egg, while that which has received the female substance alone gives rise to the male egg, while all the nuclei in which the divisions have been equal degenerate throughout the mass of cytoplasm. The evidence for this rests on the fact that in the later oöcyte stages, when the dimorphism of the eggs, male and female, is already clearly obvious, we can still distinguish the two portions of the nucleus, male and female. In the case of the female egg there are always two parts of the nucleus, while one only is shown by the male egg. The nucleus of the female egg is divided into two compartments, as in the early oögonial stages, whereas that of the male egg is single and contains only one large chromatic body.

In fig. 32 is shown on one side two almost fully mature female eggs (*ov. f.*) In this section their large centrally placed nuclei are not shown, but at the periphery a nucleus in each egg is undergoing the process of absorption. They are undergoing obvious disintegration, and stain faintly and irregularly. In both, the male and female portions of the nucleus show. Therefore those eggs in which the sex-determining division has taken place have the power of absorbing the more immature oögonial cells in which the divisions of the nucleus, male and female, have been equal, or in which sex-determination has not taken place.

In the section drawn in fig. 32, a male egg (*ov. m.*) is

shown with its single chromatic body. The nuclei of the male and female eggs can be further compared in the early stages by the examination of figs. 26 and 23.   Here in the female egg (fig. 27) the male and female portions of the nucleus are still distinguishable (*m. pro.; fe. pro.*), while only one large chromatic body is present in the male egg (fig. 26). In fig. 13 these differences are well shown in a whole preparation.   Here the large female eggs always have two conspicuous chromatic bodies, while the male eggs, which usually occupy the anterior ends or median posterior portion of the ovary, have one.   In the lower ventral portion of the ovary, amongst the small oögonial cells, are many darkly staining sperm heads.   It must be remembered that the evidence for this process of sex-determination has to be pieced together from a number of different sections, for it is, of course, impossible to follow these changes on living material and actually see the sex-differentiation that follows this division of the nucleus. The evidence of sections, as far as it goes, seems to be in favour of the view I have put forward above.

There is, however, another possible way of throwing light on the matter, and that is by experimental means. By the exclusion of the males we should expect that all the eggs should be male and of the same size.   Here again it must be kept in mind that even in the male eggs their cytoplasm has been under the influence of the sperm in all the divisions previous to the sex-determining one. Their exclusion may not result in the parthenogenetic eggs bearing characters similar in every respect to the sexual. What experimental evidence I have been able to obtain is of a doubtful nature, and for that reason I should like to defer its consideration to a future paper.

5. Maturation Stages in the Male and Female Egg.

To obtain good sections of the maturation stages many technical difficulties have to be overcome ; the large female

eggs are remarkably granular and usually break badly when sectioned. They are also difficult to handle, and the only successful method of doing this is to attach them to pieces of liver as soon as they have been fixed, and then run them through the solutions. In this manner they are not lost, and can be readily cut in numbers in small blocks. My investigation of these stages has been very slow on account of the difficulty I have experienced in obtaining the eggs in numbers. The females do their egg-laying at night or in the early hours of the morning, and the maturation stages are quickly passed through once the eggs are deposited in the sea-water; for this reason it is difficult to obtain large numbers of eggs showing the actual extrusion of the polar bodies.

In view of the peculiar nature of the male and female eggs of D. gyrociliatus, great interest attaches to the investigation of the maturation divisions, but although I have devoted a great deal of time to the matter, I have been unable to decide many points with regard to the exact number of chromosomes extruded, on account of lack of sufficient material. I have been unable yet to obtain sections of the extrusion of the second polar body in the female egg, and until recently, I have been in considerable doubt as to whether a second polar body is given off by this egg. In my preliminary paper (17) I have suggested that only one is given off and that the second is simply derived from division of the first. I have, however, since established the fact that two are given off by the female egg, but as yet I have not obtained the second in sections. My knowledge, therefore, of the maturation divisions is incomplete in one important respect. This is the case also with regard to several stages in the formation of the chromosomes after the extrusion of the polar bodies, my series not being consecutive.

In the absence of any knowledge of the number of chromosomes that go out in the second maturation division in the female egg, it is impossible to consider their significance with

regard to the peculiar form of fertilisation described in the foregoing part of this paper. In the following section the facts with regard to the maturation divisions are briefly stated, as far as I have been able to determine them up to the present, their full consideration being left for a future paper.

Korschelt (6) and Von Malsen (8) have established that in D. apatris the male and female eggs each give off two polar bodies, and this is also the case in D. gyrociliatus, as I have mentioned. The first polar body in both the male and female eggs divides again, therefore in the polar furrow of the first segmentation division three polar bodies are seen. They are extruded as soon as the eggs come in contact with the sea-water, and, as in D. conklinii, are absorbed during segmentation by one of the blastomeres.

(A) In the Male Egg.

In fig. 41 is shown a polar view of the first maturation spindle in the male egg. Twenty chromosomes are shown—the full somatic number. Twenty go out, and twenty remain in the egg (fig. 39).

In the second polar body in the male apparently ten double or dumb-bell shaped chromosomes go out and the same number remain in. In the first segmentation division of the male egg the full somatic number, twenty, is shown again (fig. 46). The full somatic number seems to be established through the breaking down of the ten double chromosomes remaining in the egg after the second maturation division (fig. 43). Each of these ten chromosomes breaks down into a special nucleus, and in a stage like that shown in fig. 43 each of the small nuclei contains two chromosomes.

In the maturation stages of the male egg a peculiar body is more or less constantly present (figs. 38, 39, and 45, pr. b.). This structure seems to have nothing to do with the process of maturation, and would seem to correspond with the so-called "besondere" body of many investigators. It usually presents the appearance of a dark, irregularly staining body

of no definite structure, as shown in figs. 38 and 39. In the first segmentation division of the male egg this body moves over without division into one of the blastomeres and then breaks up and disappears. It is not present in the female egg.

(B) In the Female Egg.

In the first maturation spindle of the female egg (fig. 37) twenty chromosomes are clearly shown. Here, again, twenty apparently go out and twenty remain in the egg, for certainly, as shown in fig. 37, eighteen or nineteen chromosomes can be counted at each end of the spindle, but as the spindle approaches the animal pole of the egg, these chromosomes apparently undergo a certain amount of fusion, and when the polar body itself is extruded, ten double or dumb-bell shaped chromosomes pass out as shown in figs. 47 and 40. These then break down into a series of blebs, which take up a central position in the egg as shown in figs. 52, 56, and 58. It is remarkable that in the female egg the number of small nuclei into which the chromosomes break down is remarkably constant (fig. 60). I have already said that I have been unable to obtain the second polar body in sections. In the spindle of the first segmentation division of the female egg twenty chromosomes are seen again at either pole (fig. 62).

6. Conclusions and Summary.

The most singular feature of the foregoing work is the peculiar manner of early fertilisation and the subsequent division of the sperm in the oögonial cells. This would seem to be without parallel, although something similar possibly takes place among the Rotifers, where we know from the work of Whitney (20) that the eggs undergo part of their growth in the presence of the sperm. I have pointed out in my paper on Histriobdella (16) that here also the sperm nucleus is often found in eggs that are far from fully formed and are only about half the size they will subsequently attain

when they are discharged by the female. In this case the sperm nucleus does not attempt to fuse with the female nucleus until this has undergone reduction, which only takes place when the eggs are laid and have come in contact with the sea-water. I was not able to determine the exact stage at which the sperm enter these eggs in Histriobdella, but it is obvious that the condition in Dinophilus is simply a more extreme example of the same thing, where the sperm join the female germ-cells as soon as these arise in the ovary.

The maturation divisions, again, in the case of the female egg of Dinophilus, only take place after, not before, fertilisation, which seems contrary to all known rule. In the case of many parthenogenetic eggs, however, we know that, although maturation divisions take place, these are not accompanied by an actual reduction in the number of chromosomes, so that their presence in these eggs would seem as unnecessary as in Dinophilus, where, if there is any reduction, it takes place after fertilisation. In the face, however, of these startling facts of early fertilisation and maturation after fertilisation in the case of the female egg, I have long delayed the publication of the present work, and have repeated all my observations again and again in the hope of arriving at conclusions more in keeping with orthodox tradition.

I know that it will be pointed out that the small accessory chromatic body of the oögonial cells, which I identify as the sperm head, is remarkably like the peculiar accessory nucleus that is so frequently present during the oögonial and oöcyte stage of many eggs, and which have been so well figured in the papers of Obst (12) and Bambeke (1). The fact, however, that in Dinophilus these bodies never attain the size or shape of true accessory nuclei, and are within the nucleus and never in the yolk or cytoplasm, seems to prove that they are not of this nature or related in any way to the large crescentric yolk nuclei on many eggs. Their staining reaction, again, is always similar to that of the female chromatic substance, while their absence from the oögonial cell when early ferti-

lisation is prevented seems to effectively prove their spermatic origin.

With regard to maturation divisions, I am forced to admit that their evidence is very puzzling, and I am quite unable to explain them at present. In the case of the male egg, we should expect, as this has not been fertilised, and is therefore in a sense developing parthenogenetically, it should agree with the development of the male parthenogenetic egg of Rotifers and other forms, where they develop in the n condition, where n represents the reduced number of chromosomes. This is certainly not the case in Dinophilus, where the male egg, after reduction (if any reduction takes place), possesses apparently the full $2n$ number. It is, however, possible that the male egg of Dinophilus is similar to that of Phylloxerans, which develops in the $2n - 1$ or $- 2$ condition, as it is so difficult to accurately count the chromosomes in the segmentation divisions of the male egg on account of their small size. In the female eggs we should expect them, since they have been fertilised before reduction, to be in the $2n + n$ condition, if we consider the sperm to bring in the n number. In the first maturation division of this egg we should find at least thirty chromosomes, whereas their number is somewhere about twenty. On the other hand, if we suppose the female germ-cells to be in the n condition when they appear in the ovary, then after fertilisation they should show the $2n$ number of chromosomes, which agrees with the facts, but does not explain how the male egg, which has not been fertilised, is nevertheless in the $2n - 1$ or $- 2$ condition. Therefore, from whichever point of view we choose to regard it, there is no way of bringing the facts of the maturation divisions into line at present.

The following is a summary of my conclusions :

(1) In D. gyrociliatus the females are fertilised inside the egg-capsules by the males.

(2) When the female germ-cells arise in the ovary they early enter into relation with the sperm, so that the nucleus

of the oögonial cells is of a double nature, one portion of which is derived from the sperm, while the other is derived from the egg.

(3) In the nuclear divisions that follow the growth and fusion of the oögonial cells the two portions of the nucleus divide equally.

(4) In some of these divisions the female portion of the nucleus divides before the male and passes over wholly to one of the nuclei; thus one of the nuclei comes to possess half the female portion of the original chromatic substance together with the whole of the male portion, whilst the other, has only half the original female portion. This appears to be the sex-determining division. All the other nuclei of the oögonial mass in which this division has taken place then degenerate. The one which possesses only the female portion of the chromatic substance gives rise to the male, whilst the other, possessing the whole of the male in addition to the female portion, gives rise to the female.

(5) In the early male and female eggs, the dimorphism of which is already marked, these two portions of the nucleus still remain distinct. The male egg possesses only a single nucleus, while the female has a double one.

(6) During the late development of the female egg these two portions of the nucleus fuse beyond recognition.

(7) Maturation takes place after the fusion of the male and female chromatic substance in the female egg and not before.

(8) There are twenty somatic chromosomes in the male and female.

(9) Two polar bodies are given off by both the male and the female egg. The first in each case dividing after being given off.

(10) In the female egg twenty chromosomes go out in the first polar body and twenty remain in the egg; actually, ten double chromosomes go out and ten double chromosomes remain in the egg. In the male egg the same thing takes place. In the second polar body, in the case of the male egg

ten double chromosomes go out and ten remain in the egg, the ten chromosomes remaining in the egg giving rise to the full number again in the first segmentation division.

(11) In the first and second segmentation divisions of the male and female egg twenty chromosomes are present.

(12) In D. gyrociliatus the presence of large eggs, which invariably give rise to females, seems to be due to fertilisation, the unfertilised eggs being smaller and giving origin to the males.

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EXPLANATION OF PLATES 30–34,

Illustrating Mr. Cresswell Shearer's paper on " The Problem of Sex Determination in Dinophilus gyrociliatus."

LETTERING.

an. Anus. *cent.* Centrosome. *cl. r.* Ciliated rings. *e.* Eye. *fe. pro.* Female pronucleus. *g.* Gut. *m.* Mouth. *m. pro.* Male pronucleus. *mu. gl.* Mucus glands. *me. ten.* Median hairs or tentacles. *neph.* Nephridia. *œs.* Oesophagus. *ov.* Ova. *ov. f.* Female eggs. *ov. m.* Male eggs. *ph.* Pharynx. *pr. b.* Problematic body. *pr. b. l.* First polar body. *pr. b.²* Second polar body. *sol.* Solenocytes of nephridia. *sl. g.* Salivary gland. *spr.* Sperm. *st.* Stomach. *tes.* Testis.

PLATE 30.

Fig. 1.—Adult female with eggs, showing the ciliated rings in the full-grown condition.

Fig. 2.—The same showing the mucus cells underlying the ciliated bands.

Fig. 3.—The same showing the distribution of the five pairs of solenocyte-bearing nephridia.

Fig. 4.—Half-grown female showing the condition of the ciliated rings at this stage. The second and third are markedly incomplete dorsally. Ovary undeveloped. Small mass of sperm shown between stomach and gut at point where the primitive oögonial cells will arise later.

Fig. 5.—Female, younger than that of fig. 4. Showing the five pairs of nephridia and sperm mass beneath the gut.

Fig. 6.—Young female some hours after leaving the egg-capsule. Showing small mass of sperm in the gut region.

Figs. 7 and 8.—Rudimentary males.

Fig. 9.—Young female considerably older than that shown in fig. 4. Ovary partially developed, showing a mass of sperm crowded into upper part.

Figs. 10 and 11.—Rudimentary males.

Fig. 12.—Female in act of laying eggs. Shows the method by which the capsule is secreted from the mucus cells.

Fig. 13.—Ovarian region in a half-grown female. Showing male and female eggs partially formed, and sperm scattered throughout the ovary. Male and female nucleoli at this stage clearly distinguishable in the larger eggs.

Figs. 14 and 15.—Rudimentary males.

PLATE 31.

Figs. 16–19.—Small oögonial cells undergoing division; male and female portions of the nucleus shown dividing simultaneously. Fixed in sublimate-acetic.

Fig. 20.—Whole preparation of the ovary of very young female, showing the primitive oögonial cells. The ovarian tissues are crowded with sperm. One of these has already attached itself to each of the primitive eggs. Stained with aceto-carmine.

Fig. 21.—Cross section in the ovarian region of fully grown female, showing the formation of the eggs. Large female eggs clearly dis-

tinguishable with male and female pronucleus. Fixed sublimate-acetic.

Fig. 22.—Mass of yolk material from ovary, formed as the result of the fusion of a number of oögonial cells. Their nuclei are shown around the periphery of the mass undergoing active division. Male and female portions of these nucleoli clearly distinguishable. Sublimate-acetic.

Fig. 23.—Fully formed female egg from ovary, in which the two portions of the nucleus, male and female, can still be distinguished. Sublimate-acetic.

Fig. 24.—Aceto-carmine preparation of a portion of the ovary of young female, somewhat older than in the stage shown in fig. 20. The primitive oögonia are somewhat larger, and the sperm that are attached to them are losing their tails.

Fig. 25.—Section through the ovarian region of a young female, just after leaving the egg-capsule. No trace of the female germ-cells can be distinguished, and only the mass of sperm which the female has received from the male before leaving the egg-capsule is distinguishable.

Fig. 26.—Ovarian male egg usually much more granular than the female egg and with only usually one nucleolus. Sublimate-nitric.

PLATE 32.

Fig. 27.—Immature partially formed female egg showing two portions of nucleus. Sublimate-acetic.

Fig. 28.—Yolk mass from ovary showing two nuclei, each showing male and female chromatic bodies. Sublimate-nitric.

Fig. 29.—Nucleus of female ovarian egg, showing its division into male and female portion. Sublimate-acetic.

Fig. 30.—Mitotic figures in first segmentation division of female egg. Stained Lithium carmine and Lyons blue.

Fig. 31.—Ovarian egg formation, showing division of two cells, in one of which male and female portions of the nucleus have divided equally, while in the other the male portion has gone wholly over to one side. Sublimate-nitric.

Fig. 32.—Section through the ovarian region of a ripe female, similar to fig. 21, showing the eggs crowded together. Fixed in sublimate-nitric.

Fig. 33.—Section of ovarian eggs similar to fig. 31. Showing in one cell the male and female portions of the nucleus equally divided, while the other shows the male nucleus only on one side. Sublimate-nitric.